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BRONCHOVASCULAR EFFECTS OF CIGARETTE SMOKE

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ABSTRACT

This Progress Report is being prepared five months prior to the termination of a two-year grant. The conclusions previously reported in a Progress Report submitted April 15, 1972, are as follows:

(a) There are no abnormalities in the bronchial blood vessels in dogs, rats and hamsters repeatedly exposed to cigarette smoke for 4 to 10 weeks. This conclusion was based on measurement of bronchopulmonary blood flow in the dog and on examination of plastic casts of the bronchial vessels of rats and hamsters.

(b) The antitrypsin activity of blood draining the bronchial circulation was found to be equal to that of the systemic blood. The daily inhalation of cigarette smoke by male rats for 10 weeks caused an increase in the level of antitrypsin activity in the blood. There were no functional changes indicating pulmonary emphysema, although rats treated with papain and subjected to tracheoconstriction developed emphysema. The simultaneous administration of cigarette smoke to such rats did not exaggerate the functional changes. In the same series of experiments the rats with pulmonary emphysema experienced a reduction in the level of antitrypsin activity. If a deficiency in this antienzyme relates to the formation of pulmonary emphysema in rats as it does in man, there is no contributing factor due to inhalation of cigarette smoke, since its inhalation caused an elevation in the level of antienzyme activity.

The additional observations completed during the period of April 15, 1972, to January 31, 1973, which are described in this report, are as follows:

(c) Rats that have pulmonary congestion induced by paraquat show an

elevation of antitrypsin activity of the blood that is not dependent on the adrenal gland. The excised lungs of these animals also show a deficiency in total phospholipid content. Among the 10 phospholipids separated and isolated from the lung extract, the following show a reduction: phosphatidyl choline or lecithin, phosphatidyl ethanolamine, sphingomyelin and lysophosphatidyl choline or lysolecithin. The investigation of the influence of chronic exposure to cigarette smoke on the concentration of each phospholipid is in progress.

(d) It has been possible to measure mechanical properties of the lungs of anesthetized mice. The ICR strain mice have higher values of pulmonary resistance than the Swiss strain. When the ICR strain was exposed twice daily for 5 weeks to smoke generated from low-nicotine cigarettes, there was no change in functional residual capacity, indicating no sign of pulmonary emphysema. However, an increase occurred in pulmonary resistance and a decrease in tidal volume, showing that exposure to cigarette smoke causes chronic bronchospasm.

(e) The Swiss strain mice responded differently from the ICR strain to cigarette smoke. Exposure to low-nicotine cigarette smoke did not increase pulmonary resistance but exposure to high-nicotine cigarette smoke caused an increase. Exposure to either type of cigarette smoke produced a decrease in functional residual capacity. The significance of this reduction is under investigation for the remaining 5-month period during which this grant is in force. Histological examination will be used to determine whether the reduction in functional residual capacity represents pulmonary fibrosis. The development of emphysema indicated by an increase in functional residual capacity can be excluded.

I. ANTITRYPSIN ACTIVITY OF THE BLOOD AND PHOSPHOLIPID CONTENT OF THE LUNG OF RATS

A continuation of the experiments on the rat consisted of measurement of antitrypsin activity of the blood and analysis of phospholipids in the lung. The results are as follows:

A. Antitrypsin activity

Since inhalation of cigarette smoke produced an increase in antitrypsin activity of the blood, it became important to study another procedure known to produce lung damage. Pulmonary congestion was induced in rats by injection of paraquat (a weed killer), which is known to reduce surfactant activity (Cambar and Aviado, 1970). Paraquat (10 mg/kg) was administered intraperitoneally and 48 hours later the rat was anesthetized and blood collected for analysis of antitrypsin activity according to the technique of Erlanger et al. (1961). The results are summarized in Table 1.

The induction of pulmonary congestion by paraquat is accompanied by an increase of antitrypsin activity in the blood. Previous adrenalectomy did not prevent the elevation of antitrypsin activity in response to paraquat. The phenomenon is unrelated to the adrenocortical axis and is not a nonspecific response to stress. The results further suggest that procedures exerted directly on the lung in the form of either congestion (by paraquat) or inhalation (of cigarette smoke) cause an elevation of antitrypsin activity in the blood. One possible intermediate mechanism to explain the end-result is the surfactant activity or phospholipid content in the lung.

(Table 1 appears on the next page.)

Table 1. Effect of paraquat on antitrypsin activity of blood in rats.

Procedure	No. of Rats	Antitrypsin activity Mean ± SE (mg trypsin inhibitor/ml serum)
Control	5	1.56 ± 0.033
Paraquat (10 mg)	7	1.93 ± 0.092*
Adrenalectomy	3	1.58 ± 0.018
Adrenalectomy followed by by paraquat (10 mg)	3	1.81 ± 0.119*

* $p < 0.05$ compared to control rats.

Table 2. Effect of paraquat on total phospholipid content of lung of rats.

Procedure	No. of Rats	Body Weight Mean ± SE (g)	Total Phospholipid Mean ± SE (mg/g wet lung)
Control	4	202.5 ± 8.5	19.15 ± 0.73
Paraquat (10 mg/kg)	4	187 ± 6.6	16.75 ± 0.99*
Paraquat (20 mg/kg)	4	185 ± 6.5	15.25 ± 0.42*.

* $p < 0.05$ compared to control rats.

B. Assay of total phospholipids

Three groups of rats were used, as follows: 4 rats received 10 mg/kg paraquat intraperitoneally, and 4 rats 20 mg/kg paraquat by the same route, while 4 rats were controls. After 48 hours the rats were anesthetized with a mixture of urethane (200 mg/kg) and allobarbital (50 mg/kg) prior to sacrificing the animals for analysis of the lungs. The lungs were minced and homogenized with chloroform-methanol (2:1) mixture and the lipids were extracted by a method described by Folch, Lees and Stanley (1957). The homogenate was filtered through filter paper into a 25-ml graduated glass cylinder and its volume was adjusted to 20 ml. The crude extract was mixed thoroughly with 4 ml of 0.05% Ca Cl solution. The mixture was allowed to separate into two phases. After the upper phase was removed, the inside glass wall and the surface of the solution were made clear by the use of the pure solvent upper phase. The resulting lower phase was diluted to 20 ml by the addition of chloroform-methanol (2:1) mixture.

Twenty-five µl of the extract from the homogenized preparation were digested by heating at 180° C for 30 min in the aluminum heating block, following the addition of 0.6 ml of 70% perchloric acid. Then water (3 ml), 2.5% ammonium molybdate (0.5 ml) and 10% ascorbic acid solution (0.5%) were added. Color was developed by heating for 5 min in boiling water (at 95° C) according to the method of Rouser, Fleisher and Yamamoto (1970). Optical density was read at 797 mµ by the spectrophotometer. Total lipids were calculated by multiplying the phosphorus value by 25, as reported by Weinstein et al. (1969). The total phospholipis content of 3 groups of rats is summarized in Table 2 (see preceding page).

The administration of paraquat caused a reduction in the total phospholipid content. The control mean level was 19.5 mg/g wet lung; the rats that received 10 mg/kg paraquat showed a mean level of 16.75 mg/g, and those that received 20 mg/kg paraquat a level of 15.25 mg/g. The reduction in the total phospholipid content correlates with the decrease in surfactant activity of aqueous extract of the lung reported previously (Cambar and Aviado, 1970).

C. Separation and detection of each phospholipid

The phospholipids contained in the lung extract were separated by two-dimensional thin layer chromatography. Eastman silica gel chromagram sheets without fluorescent indicator were dried before use by heating in an oven for 1 hour at 75° C. Tissue lipids containing 6 or 8 μg phosphorus were applied to the sheet. The sheets were developed in the first dimension with chloroform-methanol-28% aqueous ammonia-water (65:25:3.2) mixture, and in the second dimension with chloroform-acetone-methanol-acetic acid-water (3:4:1:1:0.5) mixture in a chromatography jar (outside diameter 6 inches, height 12 inches) in a cold room at 4° C. Between runs, the sheets were dried at room temperature in a hood for 30 min and at 75° C in an oven for 1 min. The spots were detected with rhodamine 6G by a technique reported by Marinetti (1962) and Wuthier (1966). The color of the spots and their changes were observed carefully under ultraviolet light. The sheets were cut around the spots and phospholipids were extracted from the sheets with chloroform-methanol (2:1) mixture. The solvent was evaporated in an oven at 75° C in test tubes. Phospholipids were digested with 70% perchloric acid at 180° C for 30 min and the phosphorus was measured. In the analysis of the small amount of phospholipid, half the volume of the reagent was used.

The results summarized in Table 3 indicate that treatment with paraquat showed a reduction in phosphatidyl choline or lecithin, phosphatidylethanolamine and sphingomyelin. There was also an increase in lysophatidylcholine (lysolecithin), probably resulting from enzymatic conversion of phosphatidylcholine.

(Table 3 appears on the next page.)

Table 3. Effect of paraquat on concentration of phospholipids in lung of rats.

Procedure / Phospholipids	Control (4 rats) % of Total Phospholipid	Control (4 rats) mg/g Wet Lung	Paraquat, 10 mg/kg IP (4 rats) % of Total Phospholipid	Paraquat, 10 mg/kg IP (4 rats) mg/g Wet Lung	Paraquat, 20 mg/kg IP (4 rats) % of Total Phospholipid	Paraquat, 20 mg/kg IP (4 rats) mg/g Wet Lung
Phosphatidylcholine (lecithin)	47.9 ± 1.12	9.21 ± 0.55	46.9 ± 1.35	7.86 ± 0.48	46.8 ± 1.68	7.13 ± 0.25*
Phosphatidylethanolamine	25.9 ± 1.31	4.96 ± 0.28	24.2 ± 0.57	4.05 ± 0.27+	21.7 ± 1.91	3.32 ± 0.37*
Sphingomyelin	12.5 ± 0.34	2.39 ± 0.08	12.3 ± 0.39	2.06 ± 0.17	12.1 ± 0.46	1.83 ± 0.02*
Lysophosphatidylcholine (Lyso-lecithin)	0.63 ± 0.09	0.12 ± 0.02	1.2 ± 0.13*	0.19 ± 0.02+	1.4 ± 0.22	0.21 ± 0.03+
Phosphatidylserine	5.9 ± 0.72	1.12 ± 0.12	6.6 ± 0.77	1.09 ± 0.11	7.2 ± 1.08	1.08 ± 0.13
Phosphatidylinositol	4.1 ± 0.28	0.78 ± 0.03	4.6 ± 1.28	0.76 ± 0.21	6.6 ± 1.18	1.01 ± 0.19
Lysophosphatidylethanolamine	0.50 ± 0.28	0.09 ± 0.05	0.55 ± 0.16	0.09 ± 0.02	0.35 ± 0.15	0.05 ± 0.02
Phosphatidic acid	0.97 ± 0.49	0.20 ± 0.11	0.63 ± 0.26	0.11 ± 0.05	0.65 ± 0.22	0.10 ± 0.03
Diphosphatidylglycerol	0.88 ± 0.21	0.17 ± 0.04	0.83 ± 0.19	0.14 ± 0.03	1.43 ± 0.44	0.22 ± 0.07
Lysobisphosphatidic acid	0.63 ± 0.14	0.12 ± 0.02	0.75 ± 0.10	0.13 ± 0.02	0.95 ± 0.16	0.15 ± 0.03
Unidentified	0.15 ± 0.12	0.03 ± 0.02	1.6 ± 0.64	0.27 ± 0.11	1.13 ± 0.59	0.17 ± 0.09

* Significant difference ($p < 0.05$) compared to control.

+ $0.05 < p < 0.1$

D. Cigarette smoking and phospholipid content

After developing techniques for the separation and analysis of 10 phospholipids the next step was to return to the original problem of cigarette smoking as studied in the rat. These animals are currently being exposed to cigarette smoke daily and the investigation will be completed before June 30, 1973. At that time it is our expectation to correlate changes in blood levels of antitrypsin, the pulmonary content of phospholipids, and measurement of functional residual capacity and pulmonary compliance. We also hope to develop a theory that will show the interrelationship of these factors with the influence of cigarette smoke.

II. MEASUREMENT OF FUNCTIONAL RESIDUAL CAPACITY AND OF PULMONARY RESISTANCE AND COMPLIANCE IN MICE

It was suggested by Drs Hockett and Kreisher that some of our efforts be devoted to measurement of lung function in mice. If this were possible, then one would be more likely to obtain a breed with a genetic abnormality causing pulmonary emphysema.

The body plethysmograph, endotracheal catheter and intrapleural catheter, which were developed for the rat by Palacek and Aviado (1967), were further reduced in size for use in the mouse. After several trials, we were successful in measuring functional residual capacity, pulmonary resistance and compliance, and respiratory minute volume, which are described in the next paragraphs.

A. Measurement of functional residual capacity

Since functional assessment of pulmonary emphysema depends on measurement of functional residual capacity, the initial step was to develop a procedure that could be repeated in the same mouse. The animal was anesthetized with pentobarbital sodium (30 mg/kg) and a plastic catheter was inserted into the trachea via the oral cavity. The tip was shaped to allow a snag fit inside the lumen of the trachea. Then the mouse was allowed to rebreathe from a syringe containing 5 ml of pure oxygen. After 7 min, the concentration of oxygen in the syringe was determined by a Scholander gas analyzer. The content of nitrogen relates to the volume of air in the functional residual space in the lung, and this capacity was estimated by the following formula:

$$\text{Functional residual capacity (ml)} = \frac{x\,(a+b) - 80\,b}{80 - x}$$

a = volume of syringe (5 ml)
b = volume of tracheal catheter (0.15 ml)
x = concentration of nitrogen in syringe after 7 min rebreathing (%)

Fifty mice of the ICR strain, ranging in weight from 10 to 40 g and in age from 10 to 60 days, were anesthetized and functional residual capacity was measured. The results are summarized in Figure 1. The functional residual capacity ranged from 0.63 to 1.05 ml. The coefficient of correlation between functional residual capacity and body weight was 0.48.

Each of the 52 mice was allowed to recover from anesthesia for repetition of measurement 2 weeks later. Most of the mice died from bleeding or infection of the trachea. As experience was acquired, the incidence of death was reduced. After 2 weeks 21 mice were still alive for a second measurement. The results are summarized in Table 4. The first group of 7 mice, which had an initial mean weight of 16.7 g, had increased in weight to 25.7 g 2 weeks later. The functional residual capacity was unchanged, with mean values of 0.79 ml and 0.82 ml respectively. The second group of 7 mice were older than the first: the mean weight of 26.7 g had increased 2 weeks later to 32.3 g. There was a reduction in functional residual capacity from 0.85 ml to 0.76 ml after 2 weeks. In the third group, consisting of 5 mice, each showed a reduction in body weight, indicating that these animals had not completely recovered from the first catheterization. There was a fall in functional residual capacity from 0.87 ml to 0.72 ml, a change which was statistically significant.

The death and loss of body weight of some mice following the first

tracheal intubation discouraged us from continuing the repeated measurement of functional residual capacity. We are developing a less traumatic method of insertion of the tracheal catheter. Until this is available, we shall continue our investigation on the basis of a single measurement.

(Figure 1 and Table 4 appear on the next pages.)

Fig. 1. Body weight and functional residual capacity of 50 mice.

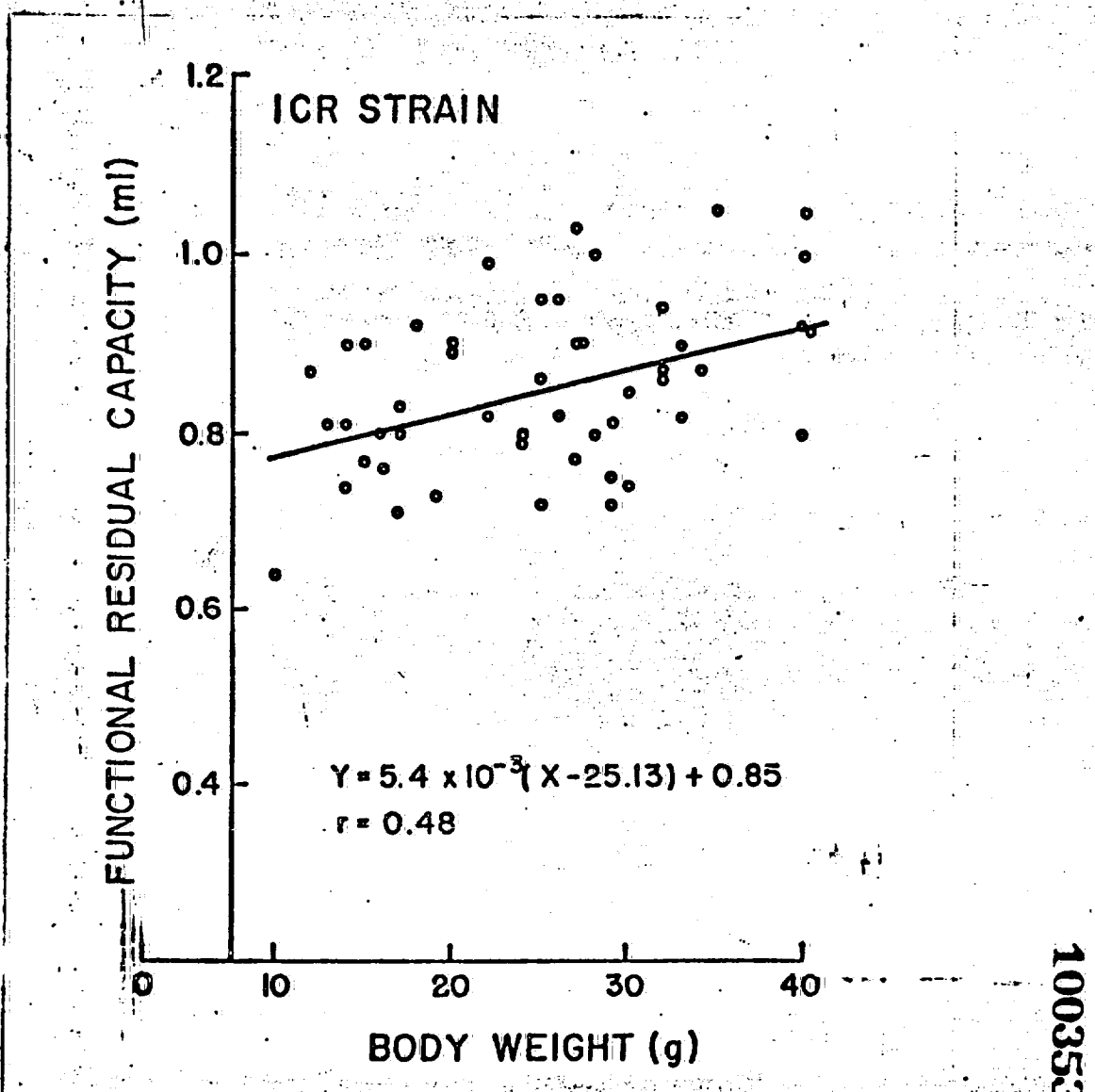


Table 4. Repeated measurements (2-week interval) of functional residual capacity (FRC) of ICR strain of mice.

Mouse No.	First Measurement		Second Measurement	
	Body Wt. (g)	FRC (ml)	Body Wt. (g)	FRC (ml)
1	13	0.81	22	0.82
2	16	0.76	27	0.75
3	17	0.80	24	0.73
4	17	0.83	26	0.83
5	17	0.71	28	0.95
6	18	0.92	27	0.73
7	19	0.73	26	0.94
Mean	16.7	0.79	25.7*	0.82
± SE	± 0.71	±0.026	± 0.78	±0.035
8	25	0.95	29	0.74
9	25	0.86	35	0.98
10	26	0.82	32	0.78
11	27	0.66	33	0.65
12	27	0.90	34	0.64
13	28	1.00	30	0.82
14	29	0.75	33	0.74
Mean	26.7	0.85	32.3*	0.76
± SE	± 0.57	±0.044	±0.81	±0.043
15	26	0.95	24	0.66
16	30	0.85	28	0.72
17	32	0.82	33	0.74
18	32	0.87	28	0.76
19	34	0.87	29	0.70
Mean	30.8	0.87	28.4	0.72*
± SE	± 1.4	±0.021	± 1.4	±0.017

* $p < 0.05$ compared to first measurement.

B. Measurement of pulmonary resistance, compliance, tidal volume and total phospholipid content

Mice of the ICR strain, ranging in age from 10 to 80 days and in weight from 9 to 45 g, were used to measure the mechanical properties of the lung. The body plethysmograph used in the rat was reduced in size for measurement of tracheal air flow, tidal volume, transpulmonary pressure, and pulmonary resistance and compliance. The mouse was anesthetized, the trachea was exposed via a skin incision on the neck, and a plastic catheter was inserted into the trachea and secured with a ligature. After measurements of lung mechanics, the mouse was sacrificed and the total phospholipid content was measured by a technique similar to that applied to the rat (see above). The results are summarized in Table 5. The 30 mice were grouped according to the following weight groups: 9 to 15 g, 16 to 26 g, 27 to 35 g, 36 to 39 g, and 40 to 45 g. As the weight of the animal increased, there was a positive correlation with pulmonary compliance, tidal volume and lung weight, but poor correlation between body weight and pulmonary resistance. The total phospholipid content remained essentially unchanged as body weight increased.

(Table 5 appears on the next page.)

Table 5. Relationship of body weight to lung measurements of ICR strain mice.

Mouse No.	Body Weight (g)	Pulmonary Compliance (ml/cm H_2O)	Pulmonary Resistance (cm H_2O/ml/sec)	Tidal Volume (ml)	Lung Wet Wt. (mg)	Total Phospholipid (mg/g Lung Wet Wt)
51	9	0.026	2.67	0.20	100	--
52	9	0.022	2.94	0.26	97	21.4
53	10	0.029	2.77	0.29	133	18.3
54	11	0.039	2.97	0.33	126	19.2
55	11	0.033	2.67	0.33	100	19.2
56	11	0.055	2.67	0.26	118	19.8
57	12	0.029	2.45	0.30	127	19.0
Mean	10.4	0.0332	2.73	0.28	114.4	19.5
±SE	± 0.43	± 0.004	± 0.068	± 0.017	± 5.7	± 0.43
58	16	0.039	2.67	0.35	176	18.1
59	18	0.046	2.94	0.40	154	18.0
60	21	0.059	2.94	0.45	169	22.7
61	23	0.064	2.67	0.46	174	19.8
62	26	0.078	2.67	0.48	254	20.1
Mean	20.8	0.0572	2.79	0.43	185.4	19.7
±SE	± 1.77	±0.0068	± 0.066	± 0.024	± 17.6	± 0.85
63	30	0.098	2.45	0.78	236	19.1
64	30	0.098	2.26	0.78	245	21.1
65	30	0.090	--	0.60	210	24.5
66	35	0.117	2.72	0.85	240	22.0
67	35	0.117	2.67	0.78	--	--
Mean	32.0	0.104	2.53	0.76	232.8	21.57
±SE	± 1.22	±0.0055	± 0.106	± 0.042	± 7.8	± 1.12
68	37	0.133	2.67	1.00	264	22.1
69	37	0.120	2.26	0.98	255	20.4
70	38	0.130	2.67	0.98	282	20.5
71	38	0.145	2.94	0.98	267	19.5
72	39	0.130	2.94	0.91	259	19.3
Mean	37.8	0.131	2.70	0.97	265.4	20.4
±SE	± 0.37	±0.005	± 0.125	± 0.015	± 4.6	± 0.50
73	40	0.150	2.67	1.01	256	21.7
74	41	0.130	2.67	0.85	310	20.1
75	41	0.145	2.94	--	274	19.2
76	42	0.155	2.94	1.02	239	22.4
77	42	--	2.83	0.87	249	22.0
78	44	0.160	2.26	1.01	350	21.6
79	45	--	--	1.04	284	22.5
80	45	0.150	2.45	0.91	289	21.9
Mean	42.5	0.148	2.68	0.96	281.4	21.4
±SE	± 0.68	±0.004	± 0.096	± 0.030	± 12.8	± 0.41

C. Comparison between ICR and Swiss strain mice

The next step was to measure the mechanical properties of the lung of Swiss strain mice. Twenty-four mice were used, ranging in weight from 10 to 45 g. The results are summarized in Table 6. As the body weight increased, there was a rise in pulmonary compliance, tidal volume and lung wet weight. Pulmonary resistance and the total phospholipid content of the lung did not increase with the other parameters.

The results of experiments on ICR and Swiss strain mice are summarized in Figures 2 to 5. The coefficients of correlation between body weight and each of the other parameters have been calculated. There is a good correlation between body weight and each of the following factors for both strains: tidal volume, pulmonary compliance and wet weight of the lung, but poor correlation with pulmonary resistance. The following difference exists between the two strains of mice: pulmonary resistance for the ICR strain is higher than that for the Swiss strain.

(Table 6 and Figures 2 to 5 appear on the next pages.)

Table 6. Relationship of body weight to lung measurements of Swiss strain mice.

Mouse No.	Body Weight (g)	Pulmonary Compliance (ml/cm H_2O)	Pulmonary Resistance (cm H_2O/ml/sec)	Tidal Volume (ml)	Lung Wet Wt. (mg)	Total Phospholipid (mg/g Lung Wet Wt)
81	10	0.020	1.70	0.20	100	18.8
82	12	0.026	1.23	0.42	123	21.1
83	13	0.036	1.40	0.29	135	22.6
84	14	0.042	1.34	0.36	135	20.4
85	15	0.040	1.47	0.38	120	26.6
Mean	12.8	0.0328	1.42	0.33	122.6	21.9
±SE	± 0.86	± 0.004	± 0.079	± 0.039	± 6.4	± 1.32
86	23	0.078	1.40	0.45	163	20.5
87	25	0.056	1.80	0.40	210	21.7
88	26	0.070	1.40	0.65	185	22.7
89	27	0.095	1.96	0.40	220	25.0
90	28	0.084	2.04	0.65	--	--
91	29	0.084	1.60	0.65	235	24.6
Mean	26.3	0.0778	1.70	0.53	202.6	22.9
±SE	± 0.88	± 0.005	± 0.113	± 0.053	± 12.8	± 0.85
92	30	0.080	1.23	0.46	234	25.0
93	30	0.117	1.96	0.71	--	--
94	31	0.088	2.10	0.67	240	27.9
95	32	0.085	1.47	0.65	328	23.9
96	32	0.117	1.96	0.59	--	--
97	33	0.100	1.55	0.65	250	21.2
98	35	0.098	1.34	0.71	279	20.4
99	35	0.110	1.70	0.78	260	23.3
Mean	32.2	0.0994	1.66	0.65	265.2	23.5
±SE	± 0.70	± 0.005	± 0.113	± 0.034	± 14.1	± 1.04
100	36	0.120	1.84	0.59	280	24.6
101	37	0.130	1.40	0.97	311	21.8
102	38	0.170	2.04	0.97	245	23.2
103	41	0.130	1.96	1.05	310	23.5
104	45	--	--	1.00	350	20.0
Mean	39.4	0.138	1.81	0.92	299.2	22.5
±SE	± 1.63	±0.011	± 0.143	± 0.083	± 17.5	± 0.72

Fig. 2. Pulmonary resistance and body weight of I C R and Swiss strain mice.

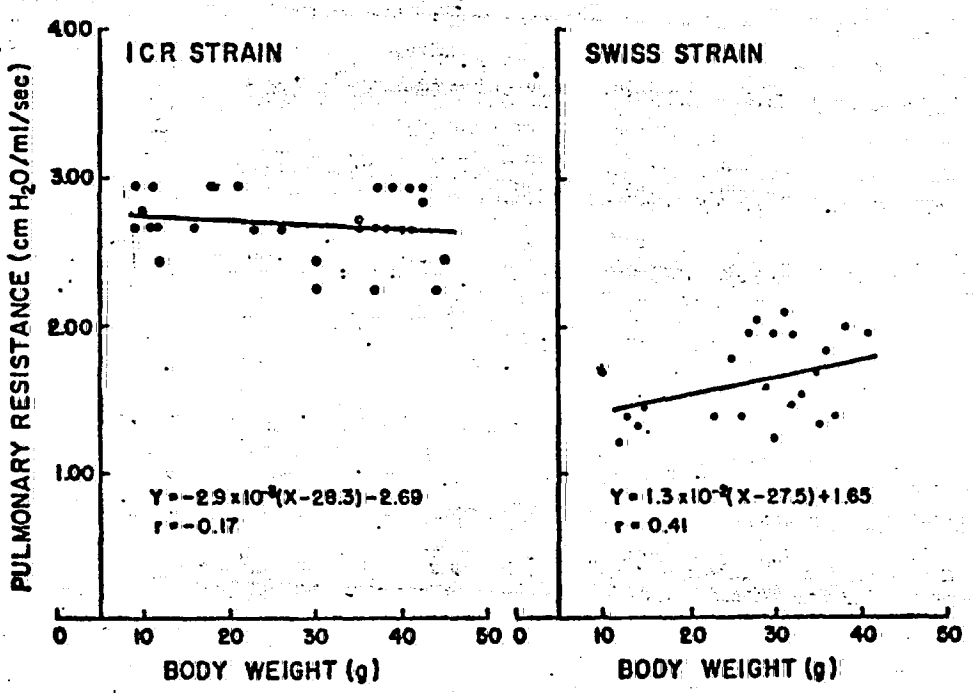


Fig. 3. Pulmonary compliance and body weight of I C R and Swiss strain mice.

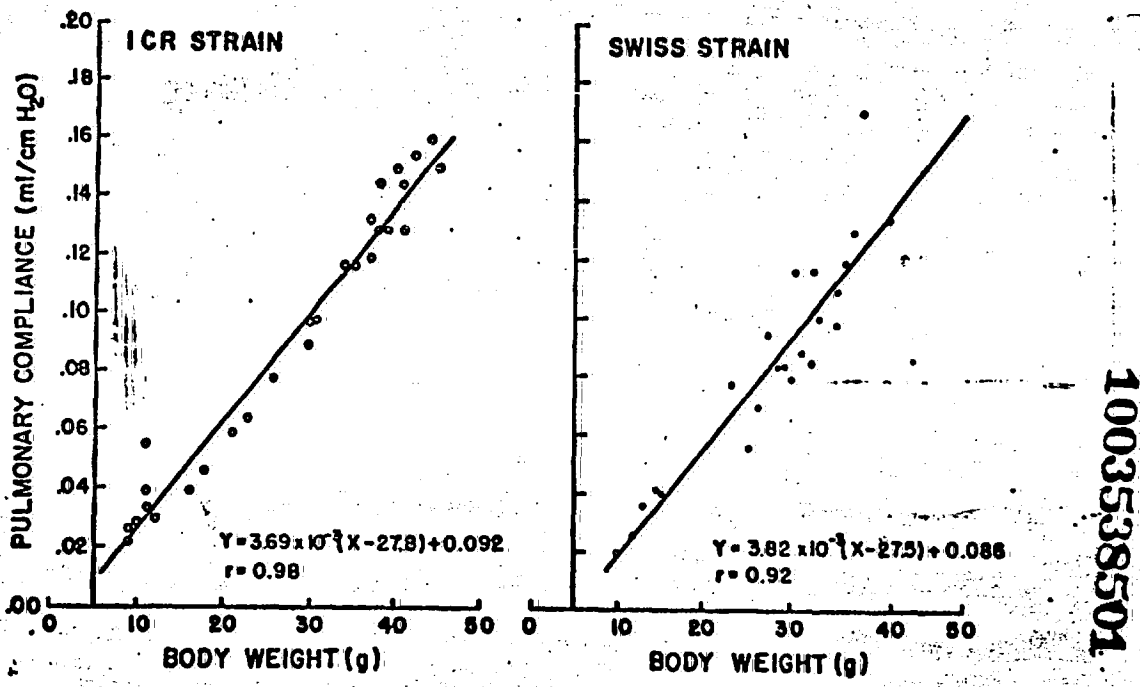


Fig. 4. Tidal volume and body weight of I C R and Swiss strain mice.

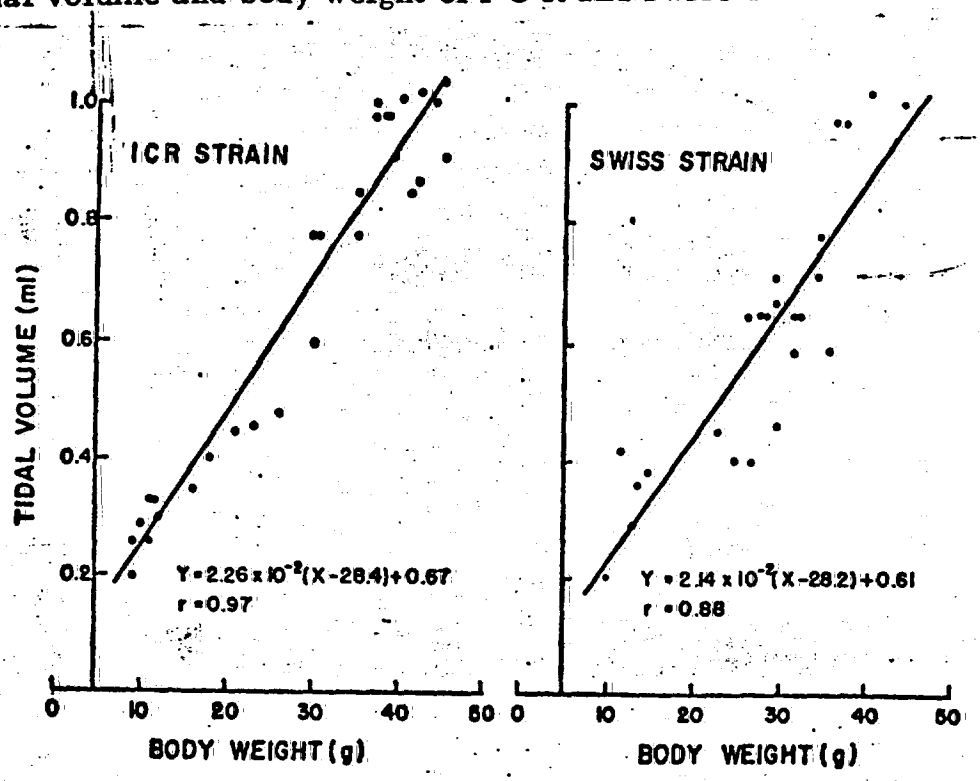
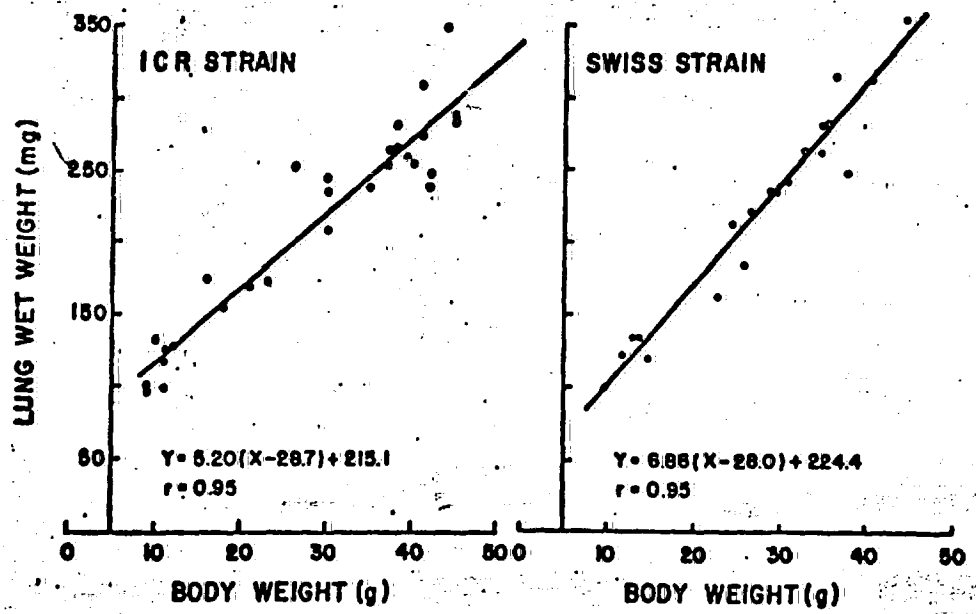


Fig. 5. Lung wet weight and body weight of I C R and Swiss strain mice.



D. Daily inhalation of cigarette smoke for five weeks by ICR strain mice

The smoking machine was used to expose mice to cigarette smoke. The mice were conditioned to the machine twice daily for 1 week. Each period of exposure to the machine lasted for 8 min. Then the mice received 8 min (or 8 puffs) of smoke generated from 2 lighted cigarettes twice daily for 5 weeks. The University of Kentucky standard cigarettes were used and were of 2 types according to nicotine content. Analyses of the leaf tobacco for the 2 types were respectively as follows:

Type I A I: 0.31% nicotine
Type I R I: 2.09% "

The initial use of the smoking machine was for a group of 8 ICR strain mice, initially weighing 25 to 30 g. These were exposed to cigarette smoke generated from IA I cigarettes, made with tobacco leaf having a low nicotine content. A control group consisting of 8 ICR mice, was not exposed to cigarette smoke. The results, summarized in Table [illegible], indicate that exposure to cigarette smoke did not influence functional residual capacity, pulmonary compliance, total phospholipid content, or the ratio of lung weight to body weight. However, there was a reduction in tidal volume and an increase in pulmonary resistance in the mice exposed to cigarette smoke, as compared with the control mice. The extent of the reduction was as follows: tidal volume from 22.0 ± 1.39 ml/kg for controls and 19.1 ± 1.3 for mice exposed to cigarette smoke; pulmonary resistance from 2.61 ± 0.057 cm H_2O/ml/sec for controls and 2.83 ± 0.148 for mice exposed to cigarette smoke.

(Table 7 appears on the next page.)

Table 7. Influence of exposure of ICR strain mice to smoke generated from low-nicotine cigarettes twice daily for five weeks.

Procedure	Mouse No.	Body Weight (g)	Functional Residual Capacity (ml)	Pulmonary Compliance (ml/cm H_2O)	Pulmonary Compliance (ml/cm H_2O/kg)	Pulmonary Resistance (cm H_2O/ml/sec)	Tidal Volume (ml)	Lung Wet Wt / Body Wt (mg/g)	Total Phospholipid (mg/g Lung Wet Wt)
Control	105	34	0.87	0.087	0.25	2.45	15	7.9	21.1
	106	33	0.88	0.078	0.24	2.83	21	9.8	24.5
	107	24	0.83	0.057	0.24	2.63	19	8.2	23.0
	108	30	0.85	0.075	0.25	2.83	26	7.4	22.0
	109	30	0.93	0.070	0.23	2.67	26	7.1	20.9
	110	30	0.85	0.078	0.26	2.45	20	7.5	--
	111	29	0.95	0.065	0.22	2.45	25	7.0	--
	112	30	0.90	0.072	0.24	2.53	24	6.4	--
	Mean	30.0	0.88	0.073	0.24	2.61	22.0	7.7	22.4
	±SE	± 1.1	±0.015	± 0.003	±0.004	± 0.057	± 1.39	±0.36	± 0.66
Low-nicotine cigarettes	113	28	1.42	0.084	0.30	2.37	23	7.3	--
	114	32	1.10	0.078	0.25	3.34	21	7.9	21.0
	115	25	0.66	0.072	0.29	2.84	13	8.0	22.4
	116	32	0.56	0.070	0.22	3.20	14	6.9	22.0
	117	33	0.92	0.052	0.16	3.20	20	6.5	21.6
	118	21	0.94	0.061	0.21	2.37	22	8.2	22.5
	119	21	0.83	0.061	0.21	2.33	19	--	--
	120	36	--	0.086	0.24	2.96	21	--	--
	Mean	28.5	0.92	0.071	0.23	2.83+	19.1+	7.5	21.9
	±SE	± 2.0	±0.11	±0.004	±0.016	±0.148	± 1.30	±0.28	± 0.28

+ $0.1 < P < 0.2$ compared to control mice.

E. Comparison of the effects of low-nicotine and high-nicotine cigarettes in Swiss strain mice

The exposure of Swiss strain mice to cigarette smoke elicited reactions that were different from those in the ICR strain. The results shown in Table 8 indicate that exposure for 5 weeks to IAI (low-nicotine) cigarettes did not influence pulmonary resistance, tidal volume, pulmonary compliance, phospholipid content or the ratio of lung wet weight to body weight. Unlike the ICR mice that showed an increase in resistance and a decrease in tidal volume, the Swiss strain mice did not show any such effect.

The measurement of functional residual capacity indicated a reduction in Swiss strain mice exposed to smoke generated by IAI cigarettes. The control group had a mean value of 1.54 ± 0.17 ml and the exposed group one of 1.03 ± 0.16 ml. The difference between the 2 groups was significant statistically ($p < 0.05$ level).

A third group of Swiss strain mice was exposed twice daily for 5 weeks to smoke generated by IRI cigarettes, which have a higher nicotine content than the IAI type. The rats exposed to high-nicotine cigarettes showed the following differences from control rats: lower functional residual capacity, higher pulmonary resistance and lower compliance if expressed in absolute terms. However, the compliance/body weight ratio was unchanged, and tidal volume, total phospholipid lung content and wet weight/body weight were not different from those of the control group of mice.

(Table 8 appears on the next page.)

Table 8. Influence of exposure of Swiss strain mice to smoke generated from low-nicotine or high-nicotine cigarettes twice daily for five weeks.

Procedure	Mouse No.	Body Weight (g)	Functional Residual Capacity (ml)	Pulmonary Compliance (ml/cm H_2O)	Pulmonary Compliance (ml/cm H_2O/kg)	Pulmonary Resistance (cm H_2O/ml/sec)	Tidal Volume (ml)	Lung Wet Wt / Body Wt (mg/g)	Total Phospholipid (mg/g Lung Wet Wt)
Control	121	30	1.30	0.066	2.2	1.96	24	7.1	20.7
	122	33	1.58	0.069	2.1	1.84	18	8.4	27.6
	123	30	1.55	0.091	3.0	2.05	24	8.1	24.6
	124	32	1.23	0.061	1.9	1.96	18	8.0	23.2
	125	28	1.65	0.065	2.3	2.19	23	7.8	21.8
	126	32	0.71	0.065	2.0	1.56	20	7.1	23.2
	127	32	1.36	0.098	3.1	1.67	18	7.0	22.7
	128	31	1.80	0.059	1.9	1.13	21	7.1	26.5
	129	33	2.15	0.065	2.0	1.55	20	6.3	25.0
	130	34	1.17	0.091	2.7	1.63	31	7.0	--
	131	29	2.99	0.056	1.9	1.23	18	--	--
	132	30	1.00	0.048	1.6	1.47	14	--	--
	Mean	31.2	1.54	0.0695	2.22	1.69	20.8	7.39	23.9
	±SE	± 0.52	±0.17	±0.0028	±0.14	±0.09	± 1.26	±0.21	± 0.74
Low-nicotine cigarettes	133	28	0.85	0.074	2.6	1.96	23	8.1	26.6
	134	31	1.15	0.065	2.1	2.10	19	8.6	20.4
	135	30	0.71	0.081	2.7	1.91	28	6.5	22.5
	136	30	0.83	0.072	2.4	1.55	32	7.3	22.5
	137	32	1.61	0.065	2.0	1.44	20	7.9	18.[illegible]
	Mean	30.2	1.03*	0.0714	2.36	1.79	24.4	7.7	22.1
	±SE	± 0.66	±0.16	±0.003	±0.13	±0.13	± 2.46	±0.36	± 1.31
High nicotine cigarettes	138	23	1.24	0.039	1.7	1.70	16	9.3	21.2
	139	26	0.73	0.085	3.3	2.45	29	7.4	25.0
	140	25	0.57	0.039	1.6	1.84	15	7.6	27.4
	141	29	0.95	0.078	2.7	2.10	18	6.2	23.9
	142	33	1.03	0.065	2.0	1.96	15	7.8	23.3
	143	26	--	0.052	2.0	2.45	22	6.9	23.7
	144	28	--	0.065	2.3	2.26	19	--	--
	145	27	--	0.065	2.4	2.45	22	--	--
	Mean	27.2*	0.90*	0.0610+	2.25	2.15*	19.5	7.5	24.1
	±SE	± 1.06	±0.12	±0.0049	±0.20	± 0.11	± 1.68	±0.42	± 0.84

* $p < 0.05$ compared to control mice.
\+ $0.05 < p < 0.1$ compared to control mice.

F. Histological examination of the lung

One lobe of each animal that has been sacrificed in the above-mentioned experiments has been removed and preserved according to the technique of Loosli et al. (1970). We are awaiting the completion of the following experiment that is still in progress: ICR mice exposed 2 x daily for 5 weeks to high-nicotine cigarettes. When the latter experiment is completed, it would allow a comparison of the reactions of ICR and Swiss strain mice in response to low-nicotine and high-nicotine cigarette smoke, administered twice daily for 5 weeks. After completion, the lung samples will be examined by an expert pathologist who will have no information on the past history of each sample.

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